UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Formetanate Hydrochloride [3-Dimethyl aminomethylene aminophenyl methyl carbamate hydrochloride]

CASRN: 23422-53-9
EPA Chem. Code: 097301
Caswell No.: 465B

FROM: George Z. Ghali, Ph.D.
Manager, RfD/QA Peer Review Committee
Health Effects Division (7509C)

THRU: William Burnam
Chairman, RfD/QA Peer Review Committee
Health Effects Division (7509C)

TO: Dennis Edwards, PM 19
Fungicide-Herbicide Branch
Registration Division (7505C)

Chief, Reregistration Branch
Special Review and Reregistration Division (7508W)

The Health Effects Division-RfD/Peer Review Committee met on March 6, 1996 to discuss and evaluate the existing or recently submitted toxicology data in support of Formetanate Hydrochloride reregistration and to reassess the Reference Dose (RfD) for this chemical.

Material available for review consisted of data evaluation records (DERs) for a chronic toxicity/carcinogenicity study in rats **(83-5 or 83-1a and -2a)**, a chronic toxicity study in dogs (83-1b), a carcinogenicity study in mice **(83-2b)**, a multi-generation reproductive toxicity study in rats **(83-4)**, developmental toxicity studies in rats **(83-1a)** and rabbits **(83-1b)**, a subchronic toxicity study in rats (82-1a) and a battery of mutagenicity studies **(84-2)**.

A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity phase of the rat study (83-1a, MRID No. 00164343, 40640901) to be acceptable, and the data evaluation record (HED Doc. No. 005785, 007317, 010298) to be adequate.

The systemic toxicity NOEL/LOEL for both males and females were considered to be 50 ppm (2.3 mg/kg/day in males and 2.9 mg/kg/day in females) and 250 ppm (12.0 mg/kg/day in males and 15.0 mg/kg/day in females), respectively, based on body weight gain depression. In females, the NOEL for plasma, brain and whole blood cholinesterase inhibition was considered to be 50 ppm and the LOEL was considered to be 250 ppm. In males, brain cholinesterase appeared to be inhibited at 10 ppm (0.45 mg/kg/day), the lowest dose level tested. Brain cholinesterase inhibition was observed in a dose-related manner.

A subchronic toxicity study in rats (82-1a, MRID No. 42664401, 42664402, HED Doc. No. 010293) was conducted on males to establish a NOEL for brain cholinesterase inhibition. In this study the chemical was tested at 1, 10, 20 and 50 ppm. Although brain cholinesterase inhibition was observed at all dose levels tested, the response was not dose-related raising questions about the adequacy of the study and the consistency of cholinesterase activity assay and measurements. Brain cholinesterase activity as a percentage of controls were 92.9, 94.4, 93.9, 91.5% for dietary concentrations of 1, 10, 20 and 50 ppm, respectively. The Committee concluded that the findings of this study neither refute nor support the findings of the chronic toxicity study, and therefore, this study should not be used for regulatory purposes. The Committee recommended that the data evaluation record be revised to reflect the Committee's position.

The Committee considered the chronic toxicity study in dogs (83-1b, MRID No. 00164341) to be acceptable and the data evaluation record (HED Doc. No. 005785) to be adequate.

In males, the NOEL/LOEL for plasma and whole blood cholinesterase inhibition were considered to be 10 ppm (0.37 mg/kg/day) and 50 ppm (1.74 mg/kg/day), respectively. In females, the NOEL/LOEL for plasma and whole blood cholinesterase inhibition were considered to be 50 ppm (9.3 mg/kg/day) and 250 ppm (98.0 mg/kg/day), respectively. Marginal depression of brain cholinesterase activity was observed at 250 ppm (8.45 mg/kg/day in males and 9.2 mg/kg/day in females), the highest dose level tested. Cholinergic signs were also observed in males and females at 50 and 250 ppm, respectively.

There was no subchronic toxicity study in dogs (82-1b) available for review by the Committee.

B. Carcinogenicity:

The Committee considered the carcinogenicity phases of the combined chronic toxicity/carcinogenicity studies in rats (83-2a, MRID No. 00164343, 40640901) and the carcinogenicity study in mice (83-2b, MRID No. 00164342, 40707101) to be acceptable and the data evaluation records (HED Doc. No. 005785, 007317, 010298) to be adequate.

The highest dose level of 250 ppm tested in the rat was considered to be adequate for carcinogenicity testing based on brain cholinesterase inhibition. The treatment did not alter the spontaneous tumor profile in this strain of rat.

The highest dose level of 500 ppm (70.0 mg/kg/day in males and 98.0 mg/kg/day in females) tested in the mouse was considered to be adequate based on body weight gain depression. The treatment did not alter the spontaneous tumor profile in this strain of mouse.

The Committee, therefore, recommended that Formetanate be classified as a **"Group E"**, i.e. the chemical is **not likely** to be carcinogenic to humans via relevant routes of exposure.

This weight of the evidence judgment is largely based on the absence of significant tumor increases in two adequate rodent carcinogenicity studies. It should be noted, however, that designation of an agent as being in Group E is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

C. Reproductive and Developmental Toxicity:

The Committee considered the reproductive toxicity study in rats (83-4, MRID No. 40411801, 40411803) to be acceptable. The data evaluation record (HED Doc. No. 006729) was considered to be adequate provided that an executive summary be included. The systemic and reproductive toxicity NOEL/LOEL were considered to be 4.5 and 23.3 mg/kg/day, respectively, based on decreases in body weight gains of females from F1 and F2a litters during gestation, statistically significant decreases in blood and brain acetylcholinesterase activity of F1 males, and consistent, though not statistically significant, decreases in blood and brain acetylcholinesterase activity in F0 and F1 females in the 250 ppm (23.3 mg/kg/day), the highest dose level tested. The NOEL/LOEL for reproductive toxicity were considered to be 4.5 and 23.3 mg/kg/day, respectively, based on decreased pup weights and viability during lactation of pups from the F2a and F2b litters of the 250 ppm group.

The Committee considered the developmental toxicity study in rats (83-3a, MRID No. 00151570) to be acceptable and the data

evaluation record (HED Doc. No. 005451, 006482, 006821, 007317) to be adequate provided that an executive summary be included. The maternal toxicity NOEL/LOEL were considered to be 1 and 3 mg/kg/day, respectively, based on statistically significant ($p < 0.001$) reductions in body weight gains for days 6-16 and 6-20 in dams and statistically significant reduction in food consumption for days 12-14 ($p<0.001$) and days 15-17 ($p<0.05$). The developmental toxicity NOEL was considered to be 5 mg/kg/day, the highest dose level tested.

The Committee considered the developmental toxicity study in rabbits (83-3b, MRID No. 00151571) to be acceptable and the data evaluation record (HED Doc. No. 005451, 007317, 000000) to be adequate. The maternal toxicity NOEL/LOEL were considered to be 5 and 15 mg/kg/day, respectively, based on reduced body weight gain and reduced food efficiency. The Developmental toxicity NOEL/LOEL were considered to be 5 and 15 mg/kg/day, respectively, based on increased incidence of 7th presacral vertebrae, increased incidence of incomplete ossification or absence of one or more cervical vertebral centra, and decreased incidence of upper and lower incisor eruption.

D. Acute and Subchronic Neurotoxicity:

There was no acute neurotoxicity study (81-8) or subchronic neurotoxicity study (82-7) available for review by the Committee.

E. Mutagenicity:

The Committee considered the following mutagenicity studies to be acceptable:

1) Mouse lymphoma L5178Y $TK^{+/-}$ forward gene mutation assay (MRID No. 00149921, HED Doc. No. 007317): Positive dose-related and reproducible increases in total mutant colonies and the mutation frequencies at 20-80 µg/mL -S9 and 40-80 µg/mL +S9. Mutagenic activity was detected at cytotoxic concentrations (≥40 µg/mL -S9 and ≥60 µg/mL +S9) and non-cytotoxic doses.

2) In vitro cytogenetics in human lymphocytes (MRID NO. 00152837, HED Doc. No. 007317). Significant and generally dose-related clastogenic effects at 60-140 µg/mL -S9. Non-activated doses ≥100 µg/mL -S9 were cytotoxic. No genotoxicity was seen in the presence of S9 activation up to the HDT (500 µg/mL) which was near the cytotoxicity limit (625 µg/mL) of the test substance.

3) Mouse micronucleus assay (MRID No. 42664405; Doc. No. 010298). The test is negative in CD-1 mice up to the HDT (9.2 mg/kg) administered once by oral gavage. Overt toxicity but no adverse effect on the target tissue (bone marrow) was seen at the HDT.

4) In vitro unscheduled DNA synthesis (UDS) assay in HeLa cells (MRID No. 40883201; Doc. No.007317): The test is negative over a concentration range that included cytotoxic doses (≥960 µg/mL -S9; ≥1920 µg/mL +S9).

In addition to the above studies, there were other open literature studies. Formetanate has been included in published genetic toxicology screening batteries of pesticides designed to correlate chemical structure to genotoxic activity (Garrett, N.E. et al., 19--; Klopman, G. et al., 1985). Qualitative results indicate that Formetanate is not mutagenic in bacterial systems.

Overall, the Committee concluded that non-activated and S9-activated Formetanate Hydrochloride is a confirmed mutagen for mouse lymphoma cells; it is also a confirmed clastogen for cultured human lymphocytes but only in the absence of S9 activation. The evidence of clastogenic activity only under non-activated conditions as compared to positive responses with and without S9 in the gene mutation assay may be related to differences in the exposure regime. In the mouse lymphoma study, cells were treated 4 hours versus a 2-hour treatment of the human lymphocytes. The marked difference in cytotoxic dose levels (≥60 µg/ML +S9--mouse lymphoma cells versus >500 µg/mL +S9--human lymphocytes) would appear to support this interpretation of the results. We conclude, therefore, that Formetanate Hydrochloride exerts a genotoxic effect on mammalian cells in vitro. However, the negative data from the micronucleus assay suggest that this intrinsic mutagenic potential is not expressed in vivo. This assumption is further supported by the lack of an oncogenic effect in rat or mouse long-term feeding studies and the absence of significant reproductive or developmental toxicity attributable to a mutagenic mode of action (i.e., decreased total implants, increased resorptions).

The submitted test battery did not include an acceptable Salmonella typhimurium reverse gene mutation assay. A Salmonella typhimurium reverse gene mutation assay is, therefore, required to satisfy the new mutagenicity initial testing battery guidelines. No other genetic toxicology data are required at this time.

F. Reference Dose (RfD):

The Committee recommended that the RfD for this chemical remain unchanged. The RfD was based on a two-year rat feeding study with an LOEL of 0.45 mg/kg/day. Brain cholinesterase inhibition was observed at this level.

An uncertainty factor (UF) of 100 was applied to account for both inter-species extrapolation and intra-species variability. An additional uncertainty factor of 3 was also applied to account for the lack of a NOEL for brain cholinesterase inhibition in this study. On this basis, the RfD was estimated to be 0.002 mg/kg/day.

It should be noted that this chemical has not been reviewed by the FAO/WHO joint committee meeting on pesticide residue (JMPR) and that an acceptable daily intake (ADI) has not been established by that Committee.

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G. Individuals in Attendance:

Peer Review Committee members and associates present were William Burnam (Chief, SAB; Chairman, RfD/Peer Review Committee), George Ghali (Manager, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Albin Kocialski (Senior Science Advisor, HED), Mike Ioannou (Acting Chief, TB II), Nancy McCarroll, Guruva Reddy, Ester Rinde, James rowe, William Sette, Henry Spencer, and Rick whiting.

Scientific reviewers (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report)

Tim McMahon _____

Mike Ioannou _____

Respective Branch Chief (Committee member; signature indicates concurrence with the peer review unless otherwise stated)

Mike Ioannou _____

CC: Stephanie Irene
Debra Edwards
Albin Kocialski
Mike Ioannou
Tim McMahon
Beth Doyle
Amal Mahfouz (OW)
RfD File
Caswell File

H. Material Reviewed:

1. Mallyon, B. A. (1988). T87 Technical Formetanate Hydrochloride: An Evaluation of Dietary Oncogenic and Chronic Toxicity Potential in the Rat. MRID No. 00164343, 40640901. HED Doc. No. 005785, 007317, 010298. Classification: Acceptable data. This study satisfies data requirement 83-5 or 83-1a and 83-2a of Subpart F of the Pesticide Assessment Guideline for chronic toxicity/carcinogenicity testing in rats.

2. Mason, S. M. and Crofts, M. (1992). T97 Formetanate: Technical formetanate hydrochloride: Investigational study into the effects on acetylcholinesterase in the rat following treatment for 3 months. MRID No. 42664401, 42664402. HED Doc. No. 010293. Classification: Acceptable data. This study satisfies data requirement 83-2b of Subpart F of the Pesticide Assessment Guideline for carcinogenicity testing in mice.

3. Mallyon, B. A. (1988). 85 Technical formetanate Hydrochloride: An evaluation of dietary oncogenic potential in the mouse. MRID No. 00164342, 40707101. HED Doc. No. 005785, 007317. Classification: Core guideline data.

4. Massey, J. et al. (1986). T82-Formetanate Hydrochloride: Dietary Toxicity Study in Beagle Dogs (Final Report-repeated administration for 52-weeks). MRID No. 00164341. HED Doc. No. 005785. Classification: Core minimum data. This study satisfies data requirement 83-1b of Subpart F of the Pesticide Assessment Guideline for chronic toxicity testing in dogs.

5. Tesh, J. M. et al. (1987). T83 technical formetanate hydrochloride: The reproductive performance of rats treated continuously through two successive generations. MRID No. 40411801, 40411803. Classification: Core minimum data. This study satisfies data requirement 83-4 of Subpart F of the Pesticide Assessment Guideline for reproductive toxicity testing in rats.

6. Willoughby, C. R. et al. (1985). Technical Formetanate - HCI: Effects of Oral Administration (Gavage) Upon Pregnancy in the Rat (Teratology Study). MRID No. 00151570. HED Doc. No. 005451, 006482, 006821, 007317. Classification: Core minimum data. This study satisfies data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

7. Ross, F. W. et al. (1985). Technical Formetanate-HCl: Effects of Oral Administration (Gavage) Upon Pregnancy in the Rabbit (Teratology Study). MRID No. 00151571. HED Doc. No. 005451, 007317. Classification: Core guideline data. This

study satisfies data requirement 83-3b of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rabbits.

8. Brown, A. G. et al. (1984). Technical Formetanate Hydrochloride: Mouse Lymphoma Mutation Assay. MRID No. 149921. HED Doc. No. 007317. Classification: Acceptable data. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing.

9. Brooker, Allen J. et al. (1985). Technical Formetanate Hydrochloride: Metaphase Chromosome Analysis of Human Lymphocytes Cultured In Vitro. MRID No. 152837. HED Doc. No. 007317. Classification: Acceptable data. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing.

10. Proudlock, R. J. et al. (1991). T95-Technical Formetanate Hydrochloride: Mouse: micronucleus test. MRID No. 42664405. HED Doc. No. 010298. Classification: Acceptable data. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing.

11. Allen, J. A. and Proudlock, R. J. (1985). Technical Formetanate Hydrochloride: Unscheduled DNA Repair in Cultured Mammalian Cells. MRID No. 156202. HED Doc. No. 007317. Classification: Acceptable data. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing.

12. Klopman, R. et al. (1985). Structure-genotoxic activity relationship of pesticides: comparison of the results from several short-term assays. Mutation Research 147:343-356. MRID No. 40909201. HED Doc. No. 004948. Classification: Unacceptable data. This study does not satisfy data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing.